RESEARCH ARTICLE

The role of sexual reproduction in the maintenance of established *Zostera marina* meadows

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Abstract

1. For clonal plants, the role of sexual reproduction in the maintenance of populations can vary widely. Some species are dependent on repeated seedling recruitment. For other species, interactions between adults and seedlings within existing populations can affect seedling survival and limit sexual reproduction in existing populations. Genetic studies of seagrass populations increasingly suggest sexual reproduction is important for the resilience and stability of their populations, but as of yet little observational data support these findings. Because seagrass populations provide important ecosystem services and are threatened with increasing anthropogenic impacts, understanding their reliance on sexual reproduction is evolutionarily and ecologically important.

2. The goals of this study were to determine (a) whether seedlings of a marine angiosperm, *Zostera marina*, establish and recruit within existing *Z. marina* meadows and (b) whether interactions between seedlings and surrounding adult shoots influence the survival of established seedlings. To meet these goals, surveys estimated seedling establishment and tracked seedling survival within multiple populations. Manipulative experiments then tested the impact of neighbouring adult shoots on seedling survival and the overall trajectory of experimental plots with and without sexual reproduction.

3. A 3-year survey identified established seedlings within *Z. marina* meadows each year. Additionally, concurrent seed addition experiments indicated seed supply could influence seedling establishment rates. A survey tracking the survival of tagged seedlings, as well as the height and density of surrounding adult shoots, showed adult shoots may negatively impact seedling survival. Experiments then demonstrated that seedlings without neighbouring shoots survived longer than those with neighbouring shoots. Lastly, two transplant garden experiments comparing the survival of plots with and without seeds highlighted that seedling recruitment is likely most important to maintain bottom cover where disturbances generate gaps in the adult population.

4. *Synthesis*. This study demonstrates that seedlings do establish within existing seagrass meadows, and that some survive to recruit into the adult population. Competition with existing vegetation, however, can be a factor compromising seedling survival. Sexual reproduction may thus most likely occur in,

and be most important for, clonal plant populations that experience seasonal disturbance.

1 | INTRODUCTION

The role of sexual reproduction in the population dynamics of a species can vary widely for clonal plant species, such as seagrasses, which are capable of both sexual and asexual reproduction. Sexual reproduction in clonal plants occurs through seedling recruitment, the germination, growth and survival of seedlings into the reproductive population (Eriksson & Ehrlen, 2008). Determining the patterns and relative importance of seedling recruitment within populations is therefore critical to understand both the population dynamics and the life-history evolution of these species (Eriksson, 1989, 1993). Clonal species for which seedling recruitment is rare among adult plants exhibit 'initial seedling recruitment' (ISR) patterns with high dependence on asexual reproduction after an ISR cohort (Eriksson, 1993). Seedling recruitment within a population is low or non-existent subsequent to this initial recruitment wave. Conversely, species exhibiting a 'repeated seedling recruitment' (RSR) pattern consistently depend upon seedling recruitment among adult plants for population maintenance. These classifications of recruitment may represent the extreme endmember classification for designating the role of sexual reproduction for a given species, as populations may differentially rely on sexual reproduction for population maintenance and resilience, especially where disturbance alters competition for resources (Bullock, 2000; Eriksson, 1993; McMahon et al., 2017).

Seagrasses are ecologically important aquatic plants that are increasingly subjected to both natural and anthropogenic stresses (Orth et al., 2006; Waycott et al., 2009). As clonal plants able to reproduce sexually and asexually, reproductive plasticity may be an important trait conferring resilience to seagrass populations in the face of these pressures. Because seagrasses produce both asexual and sexual progeny, their seeds may potentially not only germinate among adult plants of the same or different species but also the asexual progeny, or ramets, of their own parent plant. The presence of adult plants surrounding their seedlings may limit the space, light, nutrients and other limiting resources available to seedlings and can therefore influence seedling survival (Bullock, 2000; Bullock & Silvertown, 2003). For seagrasses, the density and height of surrounding shoots may dictate the extent to which adult shoots exploit resources more effectively than seedlings in their vicinity (Bintz & Nixon, 2001; Ralph, Durako, Enriquez, Collier, & Doblin, 2007; Robertson & Mann, 1984; Zimmerman, 2003). The density and height of seagrass shoots can, however, change with seasons and the availability of critical resources, such as light or nutrients (Dennison, 1987; Orth, 1977; Short, 1983; Van Lent, Verschuure, & Veghel, 1995). Changes in the adult population structure could

therefore dramatically alter the interactions between existing clones and seedlings in space and time, with high densities and heights of adult shoots likely providing the most significant resource competition with seedlings during peak growth and biomass.

Zostera marina is the dominant seagrass in the temperate waters of the Northern Hemisphere. Across this species' distribution, populations invest and rely variably on sexual reproduction for population maintenance, resilience and dispersal to new areas distant from the parent plant (Jarvis, Moore, & Kenworthy, 2012; Phillips, Grant, & McRoy, 1983; Robertson & Mann, 1984). Populations with 'annual' or 'mixed-annual' life histories subsist primarily on sexual reproduction and recruit from seed annually at locations where populations collapse seasonally (Jarvis et al., 2012; Kim, Kim, Park, & Lee, 2014; Robertson & Mann, 1984; Santamaria-Gallegos, Janchez-Lizaso, & Felix-Pico, 2000). Sexual reproduction, through dormant seeds, allows these populations to re-vegetate areas once environmental conditions have improved. Conversely, populations with a 'perennial' life history rely substantially less or not at all on sexual reproduction and largely survive through asexual reproduction (Billingham, Reusch, Alberto, & Serrão, 2003; Reusch, Boström, Stam, & Olsen, 1999). Asexual reproduction can be less energetically expensive and risky than sexual reproduction and allows perennial populations to maximize clone survival and growth under favourable environmental conditions with limited disturbance (Philbrick & Les, 1996). As a result, some perennial *Z. marina* meadows growing under favourable conditions are almost entirely monoclonal (Reusch et al., 1999). Sexual reproduction does, however, occur within these populations and seeds have facilitated perennial population recovery from mass die-off events (Jarvis & Moore, 2010; Plus, Deslous-Paoli, & Dagault, 2003). Genetic analyses of perennial *Z. marina* populations within Long Island, New York also suggest seedling recruitment contributed substantially to the expansion and recovery of a perennial population (Furman, Jackson, Bricker, & Peterson, 2015). Low levels of seedling establishment within perennial *Z. marina* populations may even occur annually, but with complete (Olesen, 1999) or near total mortality, except in areas on the periphery of the denser portions of meadows or below a critical depth or disturbance threshold (Olesen, Karuse-Jensen, & Christensen, 2017). The high seedling mortality rates in existing meadows are attributed to competition with adult *Z. marina* shoots. These results suggest sexual reproduction is important for colonization of *Z. marina* populations but may not be generally important for the maintenance of existing perennial meadows. Understanding the relative contribution of sexual reproduction

and seeds to seagrass populations may be more important in the modern era, as efforts to restore seagrasses in regions where anthropogenic disturbances have been ameliorated may rely on seed-based restoration methods rather than traditional whole plant transplantation.

The goals of this study were to determine whether *Z. marina* seedlings establish and recruit within existing meadows of *Z. marina* and if interactions between seedlings and surrounding adult shoots influence the survival of these seedlings. The objectives were as follows: (a) to determine the degree to which seedlings establish within existing meadows; (b) to test whether seed availability influences the seedling establishment rate within existing meadows; (c) to quantify the relationship between seedling survival and surrounding adult vegetation and (d) to evaluate the relative influence of sexual reproduction on the maintenance of *Z. marina* meadows through space and time. This study does not explicitly test density-dependent effects of adult shoots and seedlings on one another. Instead, this study evaluates competition as the outcome of interactions between adult shoots and seedlings that may compromise seedling survival and potentially impact seedling recruitment patterns.

2 | MATERIALS AND METHODS

2.1 | Study sites

Surveys and experiments were conducted in seagrass meadows in the Chesapeake Bay region at several locations in the York River, VA (Allens Island [AI; −76.422W, 37.257N], Bena [BE; −76.4462W, 37.2540N], Sandy Point [SP; −76.3986W, 37.2636N], Goodwin Neck [GN, −76.444, 37.297N] and Goodwin Island [GI, −76.4055W, 37.2241N]), and a site located in a coastal lagoon on the Delmarva Peninsula (Spider Crab Bay [−75.820W, 37.337N]; Figure 1). All sites were shallow (<1.0 m at MLW). We define meadows as *Z. marina* populations that are persistent spatially and temporally within aerial surveys of Chesapeake Bay seagrasses (Orth et al., 2017). York River sites were fringing, persistent meadows with similar tidal and thermal regimes while the coastal lagoon site was located on a shoal area within a larger bay that is part of a large-scale seagrass restoration project.

Within Chesapeake Bay, *Z. marina* exhibits significant variability in growth and reproduction with the seasons and over small spatial scales (<5 km; Johnson, Moore, & Orth, 2017; Orth & Moore, 1986; Shields, Moore, & Parrish, 2018). Biomass of
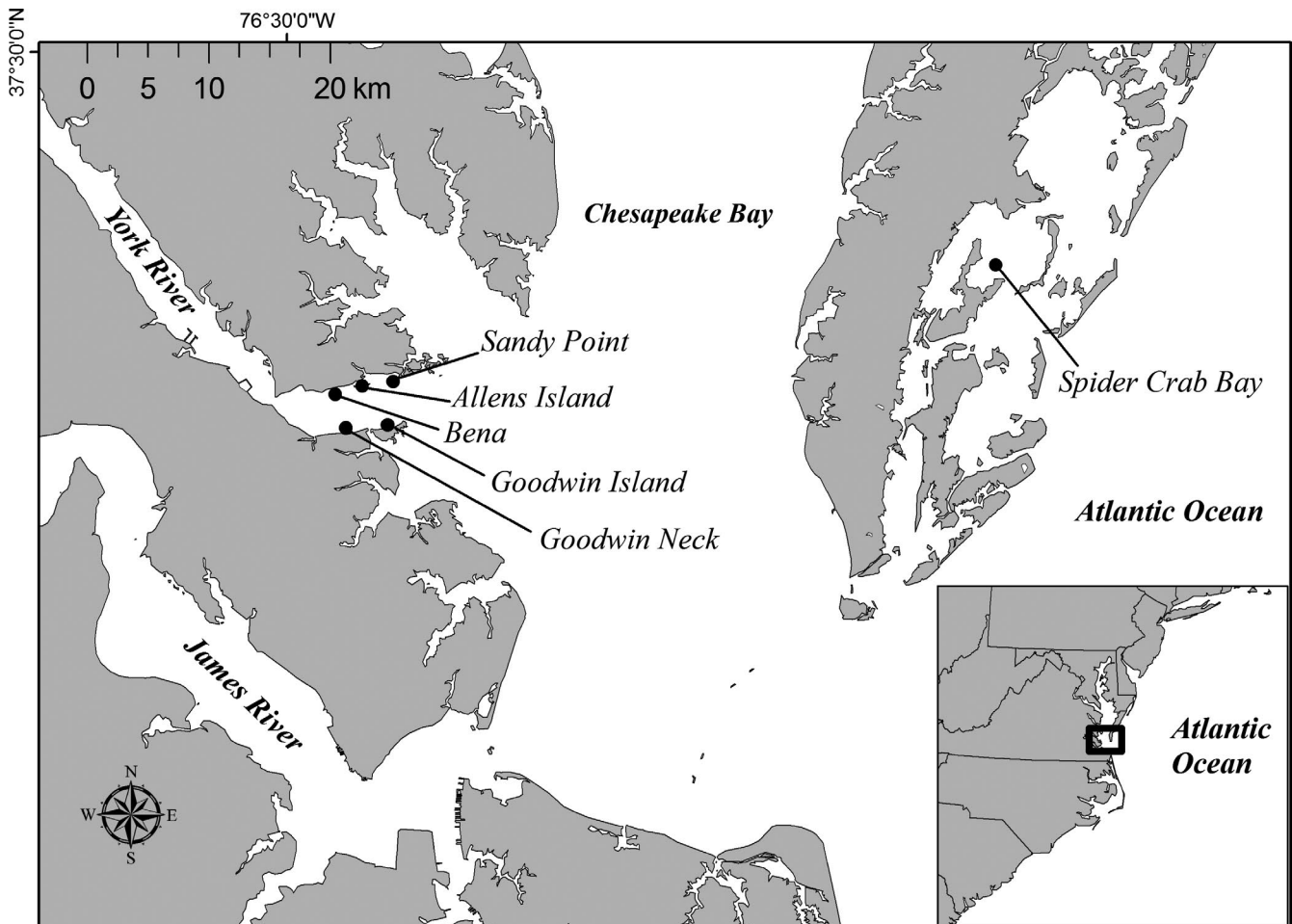

FIGURE 1 The location of experiments and surveys throughout the lower Chesapeake Bay (Bena, Goodwin Neck, Goodwin Island, Allens Island and Sandy Point) and Eastern Shore of Virginia (Spider Crab Bay)

Z. marina peaks in the spring and early summer declines dramatically during warm summer months, partially recovers during the fall, and once again senesces in the winter (Moore, Wilcox, & Orth, 2000; Orth & Moore, 1986). Flowering shoot densities can compose up to 19% of total shoots and produce up to 8,000 seeds/m^2 (Silberhorn, Orth, & Moore, 1983). Seeds remain dormant in the sediment during the spring and summer and germinate in the late fall when water temperatures drop below ~15°C (Moore, Orth, & Nowak, 1993; Orth & Moore, 1986). *Z. marina* in the region has a transient seed bank with all seeds produced in the spring germinating the following fall (Moore et al., 1993). Thus, *Z. marina* seeds in Chesapeake Bay germinate under environmental conditions favourable for growth and during a period of low adult cover and biomass. Seedlings may thus have an opportunity to germinate and grow within gaps between clones before peak biomass the following spring (here defined as 'establishment'). This growth may allow seedlings to establish carbohydrate reserves necessary to survive the most physiologically demanding warm summer months (Burke, Dennison, & Moore, 1996), and successfully recruit into the adult population the following winter (Moore, Shields, Parrish, & Orth, 2012; Silberhorn et al., 1983).

The *Z. marina* meadows of Chesapeake Bay are genetically diverse. This high diversity may be a consequence of disturbance events (e.g. hurricanes—Orth & Moore, 1983; propeller scarring—Orth et al., 2017; cownose ray activities—Orth, 1977), and large-scale diebacks within the region (Moore et al., 2012) that generate bare sediment within meadows that are often meters wide or impact an entire meadow. Populations have recovered from these large disturbances almost exclusively from seedling recruitment within the bare sediments, either from a dormant seed bank or from seeds dispersed from populations meters to kilometres from the disturbed areas (Harwell & Orth, 2002; Orth, Luckenbach, & Moore, 1994), thus providing genetic material from numerous populations (Reynolds, Waycott, & McGlathery, 2013; Reynolds, Waycott, McGlathery, Orth, & Zieman, 2012).

2.2 | Study design

This study uses a series of surveys and experiments over 3 years (2016–2018) to evaluate the influence of seed and seedling processes on perennial *Z. marina* meadows in Chesapeake Bay. Surveys and experiments were first used to evaluate natural levels of seedling establishment in the perennial meadows of Chesapeake Bay and the potential for seed supply to impact these levels. Simultaneously, experimental transplant garden plots were constructed to directly test whether the availability of seeds, and sexual reproduction, was necessary to maintain bottom cover within plots relative to plots without seeds over time. In 2017, a survey and an experiment explored the influence of surrounding vegetation on the survival of seedlings in meadows. A survey of tagged seedlings evaluated whether seedling survival was related to the characteristics of neighbouring adult shoots. Lastly,

an experimental manipulation was used to compare the survival of seedlings with adult shoots surrounding them to the survival of seedlings around whom adult shoots had been experimentally removed. We focus on seedling establishment and survival as the primary metrics of sexual reproduction in this study, as these later stages of sexual reproduction are both necessary for successful sexual reproduction but also clearly demonstrate that the previous stages of sexual reproduction (e.g. flowering, seed dispersal and seed settlement) were successful. As a result, measurements of seedling establishment and survival imply successful pollination, seed survival seed germination, and initial seedling growth in the area where individual seedlings can be observed and counted.

2.3 | Natural seedling establishment and potential seed limitation

To quantify seedling establishment within meadows of *Z. marina* and to determine whether the size of the seed bank at a given location may limit seedling establishment, in situ plots were constructed within the middle of seagrass meadows at GI, SP, AI and BE in the York River. At each location 6, 2 m^2 plots were constructed in three distinct blocks, each containing two plots, at similar depths (±10 cm) in autumn 2015. Two thousand viable seeds, collected in spring, 2015 (according to Marion & Orth, 2010), were then broadcast evenly onto the sediment surface within one, randomly selected plot in each block (three plots at each location). The remaining plot in each block did not receive additional seeds and was considered a control plot that would maintain the natural level of seedling establishment in the meadow that year. Four, 0.02 m^2 cores were taken from each 2 m^2 plot in late May 2016, 5–6 months after seed germination. The number of seedlings, the total shoot number, the maximum adult shoot height and a random adult shoot height were then recorded for each core. Seedlings were identified as having a heavily rooted and curved rhizome base (Setchell, 1929; Figure 2a). Additional seeds were added and the sampling procedure was repeated in the fall and spring of 2016–2017 and 2017–2018 (Table S1).

Because these plots were constructed within existing *Z. marina* meadows, any seedlings identified from control plots, those not receiving 2000 supplemental seeds, were likely from seeds naturally settling in that area. As such, cores taken within these plots acted as a survey of natural seedling establishment within the meadow.

A generalized linear model (GLM) fit to a Poisson distribution was used to determine whether the number of seedlings naturally establishing in meadows of the York River varied by location and/or year (Table S2). A generalized linear mixed-effects model, also fit to a Poisson distribution, was then used to evaluate if the seed addition treatment significantly increased the number of seedlings within cores taken from plots with additional seeds relative to control plots at a given location in a given year. For this model, the block, location and year of sampling were treated as nested random variables. A mixed-effects model was used to test whether mean

FIGURE 2 Seedlings and adult shoots of *Zostera marina*: (a) the distinctive hook and 'hairy' end to the rhizome used to identify seedlings in the study, (b) a seedling identified in situ with a 19 mm lock washer about to be placed around its rhizome, (c) a dead seedling dug up from the competition treatment after defoliating and (d) a mixed *Z. marina* (longer leaves, foreground) and *Ruppia maritima* (shorter leaves, middle ground) meadow at Goodwin Island [Colour figure can be viewed at wileyonlinelibrary.com]

seedling heights were significantly shorter than the mean height of surrounding, adult shoots within cores. The year and location from which shoot heights were measured were considered random variables. The heights of shoots were $\log_{10}$ transformed to meet model assumptions.

2.4 | Adult shoot neighbours and seedling survival

To evaluate whether the characteristics of surrounding adult shoots influence the survival of seedlings in *Z. marina* meadows of the lower Chesapeake Bay, seedlings were identified and followed monthly at GI, SP, AI and BE in the York River. Seedlings were identified within a single, 4 m² plot at each location in April 2017. At this time of the year, *Z. marina* seedlings have germinated, but the growth of *Z. marina* in Chesapeake Bay has not consolidated *Z. marina* meadows to the extent that seedlings are indistinguishable from adult shoots. Seedlings were identified as spatially isolated and lacking clonal integration with surrounding shoots. Once a seedling was identified, its position was recorded using 1 m² North–South orientated grid quadrats gridded into 100, 100 cm² cells that were placed over each 1 m² of the 4 m² plot. A stainless steel 19 mm washer was then slid down the leaves to the base of the shoot so that the shoot would grow through the centre of the washer and anchor the washer in place along the rhizome (Figure 2b). The 70 tagged seedlings were evaluated for survival monthly from April to October 2017 by returning to the recorded position of the seedling and gently brushing away sediment until the lock washer was visible.

The density, height and cover of adult *Z. marina* and *Ruppia maritima*, a subcanopy species co-occurring within *Z. marina* meadows, shoots were also recorded monthly in each 4 m² plot (Figure 2d). The number of *Z. marina* and/or *R. maritima* shoots within 16 haphazardly selected 0.02 m² areas was recorded within each plot, four

within each 1 m² of a plot. The length of one shoot representative of canopy height was recorded for each species per count. The density of shoots (*n* = 4) and canopy heights (*n* = 4) measured within each 1 m² subsection of the experimental area were then averaged and multiplied together. This leaf height and density metric were then multiplied by the per cent bottom cover taken for each 1 m² of the experimental area to approximate a leaf area index (LAI) per m² for each species. The four LAI estimates were then averaged to estimate a LAI for each species at each location for each month of the survey.

A first-order autoregressive model fit to a Gaussian distribution was used to test whether the mean numbers of seedlings surviving in an area was related to the mean estimated LAI of *Z. marina* or *R. maritima* shoots surrounding the seedling. The estimated LAI of *Z. marina* and *R. maritima* were considered fixed variables, but the month and the location at which seedlings were evaluated were considered nested, random variables. Due to the radically different scales between the LAI of *Z. marina* and *R. maritima*, these variables were scaled with the *scale* function in the base R package. Because bottom cover was not recorded at Goodwin Island in July, the bottom cover for that month was estimated by averaging bottom cover taken in June and August.

2.5 | Seedling competition experiment

To directly test whether adult *Z. marina* plants influence the survival of *Z. marina* seedlings within an established meadow, the survival of seedlings growing among adult shoots was compared to the survival of seedlings around whom adult shoots were experimentally removed. In all, 21 seedlings were identified and tagged with plastic-coated wire bent around the base of the shoot in May 2017 within an 11 m² area at BE in the York River, VA (Figure 2c). The location of each seedling within a North–South orientated 1 m² grid split into

100 cm^2 cells was recorded. For 10 haphazardly selected seedlings, all other shoots and rhizomes within 15 cm of the seedling were removed to eliminate any resource competition between seedlings and neighbouring shoots. The shoots surrounding the remaining 11 identified seedlings were counted and left to grow around the seedlings. After waiting 2 weeks to account for any mortality resulting from the application of treatments, the survival of each tagged seedling was recorded weekly through October 2017.

Kaplan–Meier survival curves were generated for seedlings growing with and without neighbouring, adult *Z. marina* shoots. A log-rank test was then used to compare these survival curves to determine whether the duration of seedling survival differed between these two treatments.

2.6 | Transplant garden experiments

To test whether the establishment of seed banks by sexual reproduction within existing meadows of *Z. marina* is important to maintain the bottom cover of mature *Z. marina* meadows, an experimental manipulation of adult *Z. marina* plants and seed banks was initiated in fall 2015. In all, 32 experimental *Z. marina* plots were constructed in bare sediment in eight rows of four, 1 m^2 plots at Goodwin Neck, just upstream of a persistent *Z. marina* meadow. One plot in each row was planted with (a) a known density of *Z. marina* plants (70/m^2) and *Z. marina* seeds (1,000/m^2); (b) a known number of adult *Z. marina* plants (70/m^2); (c) a known number of *Z. marina* seeds (1,000/m^2) or (d) neither seeds nor adult *Z. marina* transplants. Each of the eight rows contained one plot of each treatment. The per cent of *Z. marina* bottom cover was then evaluated monthly in each plot from May to October over 3 years, 2016–2018, to determine whether the presence of a seed bank was crucial for the long-term persistence of the plots. Seedlings of *Z. marina* and *R. maritima* detected from aerial photography in 2015 suggested this experimental area would be suitable for *Z. marina* growth. All flowering shoots were removed from plots to ensure the only sexual reproduction within plots stemmed from experimental treatments applied each autumn.

To scale up the transplant garden experiment spatially and test whether the benefits of sexual reproduction vary with location, the experimental design described above was replicated with larger plots at two locations. Sixteen 4 m^2 plots were constructed in four rows of four plots in the York River, ~35 m from the 1 m^2 plots mentioned above, and in Spider Crab Bay. One replicate of each treatment described above was haphazardly placed in each of the four rows. The density of transplants was lowered to 70 transplants per plot (4 m^2) to simulate published restoration techniques (Orth, Harwell, & Fishman, 1999). 1,000 seeds/m^2 were added to all seeded plots each autumn. Plots were constructed in fall 2016 and the bottom cover (per m^2) of each plot was evaluated monthly from May to October in 2017 and 2018.

Differences in per cent bottom cover between plots with *Z. marina* seeds, adult plants, and both adult plants and seeds were determined with first-order autoregressive models. The adult plant treatment was the referenced control for all comparisons to determine whether treatments with sexual reproduction differed in bottom cover from plots with only asexual reproduction. Bottom cover estimates were square root transformed to meet model assumptions. Analysis of deviance was then used to compare model terms. For the 1 m^2 experiments in the York River, the reproductive treatment was treated as a fixed effect while the year, month and row in which cover was evaluated were treated as nested, random effects. For the 4 m^2 experiments, the reproductive treatment and location of the experiment were treated as interacting, fixed effects while the year, month, row and quadrant in which cover was evaluated were treated as nested, random effects. Because all estimates of bottom cover during the first year may represent transplantation success more than functional survival through time, additional first-order autoregressive models were also constructed to evaluate the impact of the treatments on per cent bottom cover after the first year of growth for both experiments. Bare sediment control plots were not included in these comparisons, as these plots were used to evaluate background seedling establishment rather than for any comparison of long-term bottom cover.

All statistical analyses were performed in R statistical analysis software (R Core Team, 2018). An alpha level of 0.05 was set for all statistical tests. Coefficients of GLMs are reported as odds ratios derived from the back transformed model coefficient. Generalized linear and mixed-effect models were built with the *glm* and *glmer* functions in the LME4 package (Bates, Maechler, Bolker, & Walker, 2015). Linear mixed-effect and autoregressive models were constructed using the *lme* function from the NLME packages (Pinheiro, Bates, Debroy, Sarkar, & R Core Team, 2018). Kaplan–Meier curves and log-rank tests were conducted with the *survfit* and *survdiff* functions from the SURVIVAL R package (Therneau & Lumley, 2018). Model assumptions were assessed graphically and estimates of dispersion in GLMs were calculated manually or with the *dispersion_glmer* function in the BLMECO package (Korner-Nievergelt et al., 2015).

3 | RESULTS

3.1 | Natural seedling establishment and potential seed limitation

Naturally established seedlings were identified in control plots at all locations over the course of the three study years (Figure 3). A significant increase in the number of established seedlings was detected between plots with an additional 1,000 seeds/m^2 (Table S5; β = 2.0 ± 1.1, z = 6.7, p < .001) relative to control plots over the three study years and across all four locations. On average, more seedlings were found at GI (126 ± 65 seedlings/m^2) than at AI (55 ± 14), SP (50 ± 16) or BE (24 ± 7). More seedlings were also found in 2018 (125 ± 49 seedlings/m^2) than in 2016 (45 ± 13) or 2017 (21 ± 5). The effect of location on seedling establishment varied significantly with the year of sampling (Table S3; p < .001). All locations except GI demonstrated higher seedling establishment in 2018 relative to 2016 and

2017. Goodwin Island exhibited high seedling establishment in both 2016 and 2018. The significant interaction between the location and the year of sampling on seedling establishment likely stems from this
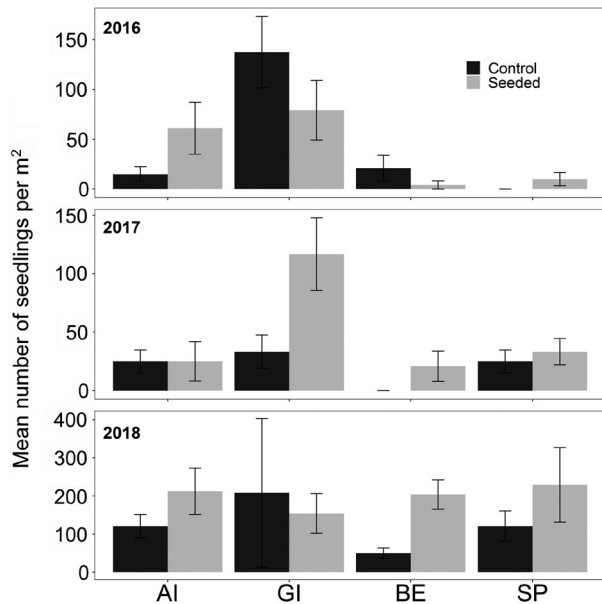

FIGURE 3 Natural seedling establishment and potential seed limitation. The mean (±*SE*) number of seedlings found in 0.02 m² cores (*n* = 12) taken from plots at sample locations in the York River from 2016 to 2018. Note the difference in scale between 2018 and the other 2 years of the study. AI, Allens Island; BE, Bena; GI, Goodwin Island; SP, Sandy Point

high seedling establishment at GI in both 2016 and 2018. Seedlings also made up the largest fraction of the total shoots present at GI (19 ± 30%) relative to SP (16 ± 26%), AI (10 ± 20%) or BE (3 ± 6%). Mean seedling heights (17 ± 0.46 cm) were consistently shorter (Table S4; β = 0.42 ± 1.1, t = −6.1, p < .001) than the mean height of surrounding vegetation (44 ± 1.7 cm) across locations. The mean difference in height between seedlings and surrounding vegetation was smaller at GI (8.5 ± 3.6 cm difference; Figure S1) than at other locations (SP: 27 ± 11 cm, BE: 30 ± 5.8 cm, AI: 40 ± 3.4 cm).

3.2 | Adult shoot neighbours and seedling survival

The mean number of seedlings present/m² was negatively related to the calculated LAI of *Z. marina* (Table S6; β = −0.09 ± 0.03, t = −3.5, p < .001) but positively related to the calculated LAI of *R. maritima* (β = 0.09 ± 0.02, t = 3.6, p < .001). The mean measured LAI for *Z. marina* was higher at BE (3,245 ± 212.8; Figure S2) than AI (1,987 ± 124.8), GI (651.6 ± 23.25) or SP (133.5 ± 17.10). The mean LAI of *R. maritima* was lower than the mean LAI for *Z. marina* and was higher at GI (430.0 ± 25) than SP (1.2 ± 0.5), BE (0.26 ± 0.13) or AI (0.068 ± 0.0039). The vast majority of seedlings disappeared between June and September 2017 (90%). Only seedlings at Goodwin Island (*n* = 6) and Bena (*n* = 1) survived into the fall growth period for *Z. marina* in Chesapeake Bay (Figure 4). Extensive algal mats appeared at SP in June 2017. All *Z. marina* and *R. maritima* within the SP plot were gone by the end of the summer (Figure S3). These mats did not occur at other study locations.

FIGURE 4 Adult shoot neighbours and seedling survival. The number of seedlings surviving during monthly sampling at Allens Island (*n* = 24), Goodwin Island (*n* = 23), Bena (*n* = 12) and Sandy Point (*n* = 11). While sampling in May 2017, an additional seedling was identified and tagged at Sandy Point. Similarly, a tagged seedling, that was not found in September, was discovered alive in October at Goodwin Island
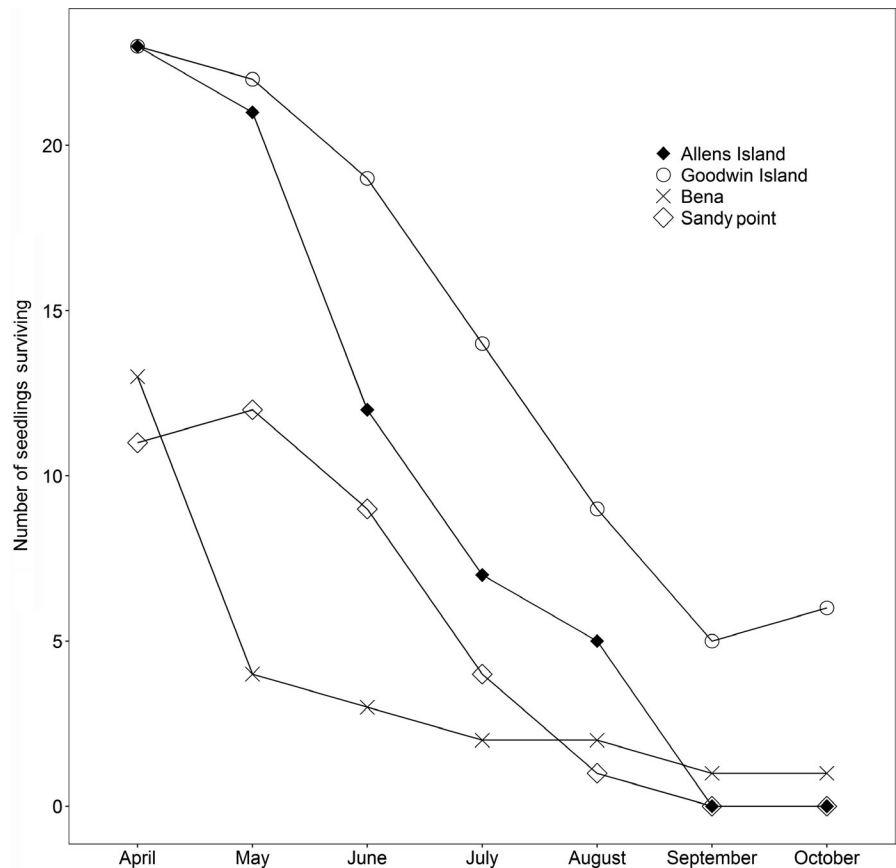
3.3 | Seedling competition experiments

The survival of seedlings without neighbouring shoots was significantly higher than the survival of seedlings with neighbouring shoots (χ^2 = 12.4, p < .001). In fact, the only seedlings to survive the experimental period were seedlings without neighbouring shoots within a 15 cm radius (Figures 2c and 5).
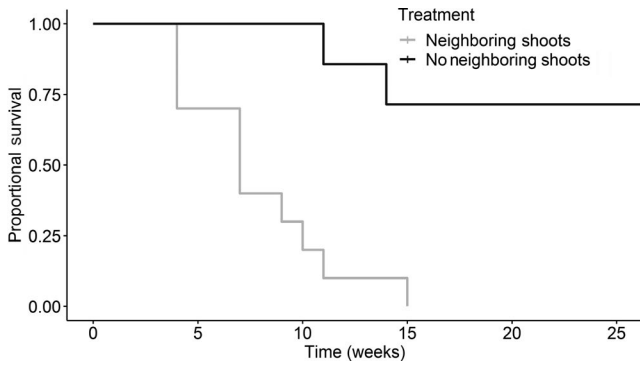

FIGURE 5 Seedling competition experiments. The proportional survival of seedlings with (grey, n = 10) and without (black, n = 7) neighbouring shoots

3.4 | Transplant garden experiments

The per cent bottom cover of plots with sexual reproduction (the transplanted and seeded or solely seeded treatments) did not significantly differ from plots with exclusively asexual reproduction (the solely transplanted treatment) across all 3 years of the study ($F_{2,286}$ = 0.68, p = .5; Figure 6). Bottom cover within seeded plots did, however, have significantly ($F_{2,190}$ = 10.6, p < .001) higher cover than transplant plots after the first year of the study (i.e. in 2017 and 2018). Per cent bottom cover changed seasonally within all 1 m^2 reproductive treatment plots in the York River. The highest mean bottom covers generally occurred around June and the lowest mean cover in September and October. By October 2018, the bottom cover of all plots in the York River had declined sharply.

The larger, 4 m^2 reproductive treatment experiment demonstrated differences in bottom cover between plots with and without sexual reproduction between locations. The effect of the seeded treatment on bottom cover interacted with the locations of the study (Table S7; $F_{2,1,005}$ = 13.3, p < .001). The experimental plots in the York River exhibited similar seasonal trends in per cent bottom cover to the adjacent 1 m^2 plots but were less stable and higher in peak bottom cover than plots within Spider Crab Bay (Figure 7). By October 2018, however, bottom cover within the York River declined dramatically relative to
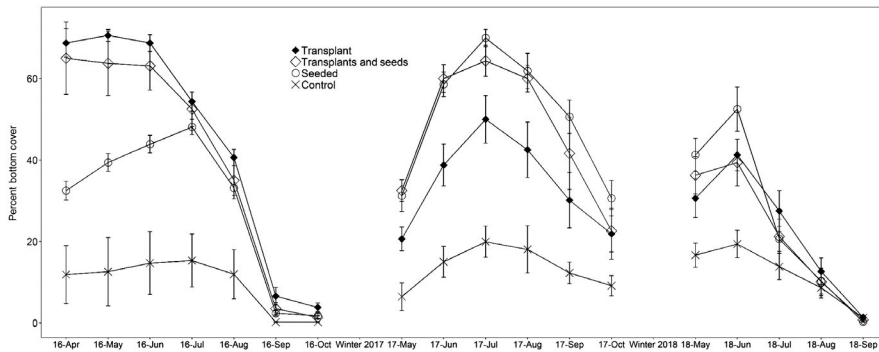

FIGURE 6 Transplant garden experiments. The mean (±SE) per cent bottom cover from 2016 to 2018 of 1 m^2 experimental plots (n = 8 per treatment) built within unvegetated sediment of the York River, VA in fall 2015
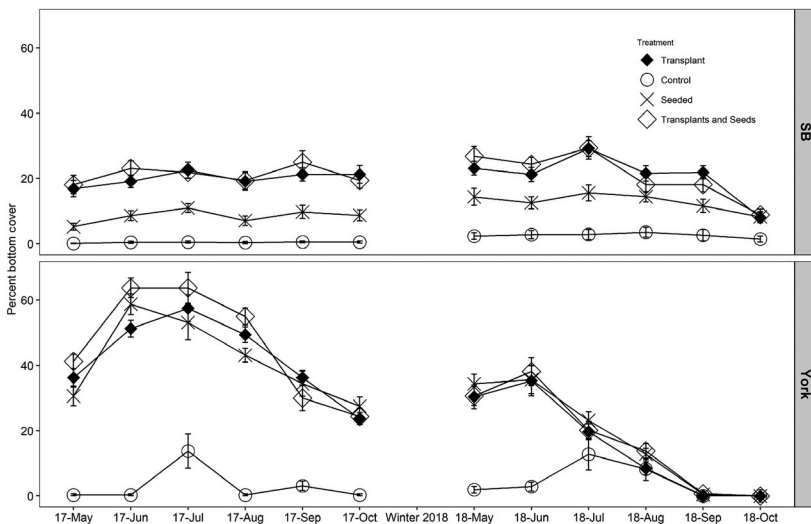

FIGURE 7 Transplant garden experiments. The mean (±SE) per cent bottom cover from 2017 to 2018 of 4 m^2 experimental plots (n = 4 per treatment) built within unvegetated sediment of the York River, VA (York) and Spider Crab Bay, VA (SB) in fall 2016

those in Spider Crab Bay. The seeded treatments, in particular, maintained higher bottom cover within the York River than the equivalent treatments in Spider Crab Bay. Interestingly, plots with adult plants (i.e. the transplanted and seeded or solely transplanted treatments) always maintained the highest cover in Spider Crab Bay.

4 | DISCUSSION

Our results demonstrate seedling establishment consistently occurs within the perennial *Z. marina* meadows of our study system, but the magnitude of establishment varies substantially over space and time. Seed availability or the size of the seed bank may partially explain the observed variability in the seedling establishment rate. Unexplored factors, such as local seed predation (Fishman & Orth, 1996) or winter storms (Jarvis & Moore, 2015; Marion & Orth, 2012), can also, however, markedly influence seedling establishment rates. Established seedlings contributed to the structure of meadows during peak biomass in this region (as high as 20% of shoots). A small proportion of established seedlings also survived to recruit into the reproductive population within surveyed meadows. Interactions between seedlings and surrounding vegetation may be one of several critical factors influencing the survival of seedlings within existing meadows. Seedling recruitment, and therefore sexual reproduction, appears to be more important in meadow maintenance at locations with regular disturbances that create patches of bare sediment within the meadow. Disturbances may potentially generate gaps in the existing population that open opportunities for greater survival of seedlings within the meadow. Sexual reproduction may thus play an important role in the maintenance of perennial *Z. marina* meadows that experience stochastic disturbances that create bare areas within established meadows.

4.1 | Role of sexual reproduction: disturbance-driven relevance

Seagrasses generally, and *Z. marina* in particular, invest in and rely variably on sexual reproduction for population survival. The relevance of sexual reproduction to population survival occurs along a continuum, from essential for annual populations, entirely dependent on RSR, to unnecessary, for perennial populations with little or no flowering, capable of surviving clonally after an ISR. Results in this study support these general findings with surveyed perennial populations of *Z. marina* in the lower Chesapeake Bay recording similar or slightly higher levels of seedling establishment to other perennial *Z. marina* populations in China (Xu et al., 2018) and the Baltic Sea (Olesen et al., 2017). Similarly, few seedlings within the perennial *Z. marina* meadows of Chesapeake Bay survived to recruit into the population as in other locations. The survival of even small numbers of seedlings among adult shoots observed in our study, however, provides strong observational support to genetic studies of clonality that conclude seedling recruitment (and thus the emergence of new genets) occurs in existing perennial *Z. marina* populations, and that sexual reproduction is important to

maintain the structure and genetic diversity of seagrass populations generally (Furman et al., 2015; Reusch, 2006; Sherman, York, Smith, & Macreadie, 2016; Sinclair, Krauss, Anthony, Hovey, & Kendrick, 2014). The observed variability across populations of perennial *Z. marina* meadows recorded in this study suggests the importance of sexual reproduction for seagrass population maintenance and resilience can change over small spatial and temporal scales. For example, higher levels of establishment and recruitment at Goodwin Island relative to other locations demonstrate perennial *Z. marina* populations may rely differently on sexual reproduction over small spatial scales (≤5 km). Similarly, higher levels of seedling establishment in 2018 relative to 2016 and 2017 suggest the importance of sexual reproduction for *Z. marina* populations in Chesapeake Bay may shift between years.

Results from transplant experiments in this study suggest the role of sexual processes in perennial *Z. marina* meadow maintenance may depend on the extent to which disturbance, associated in this case with diebacks from high summer water temperatures, impacts an existing meadow. Within the York River, plots receiving seeds performed better than plots without seeds only after years with substantial summertime declines in overall cover (i.e. cover was higher in seeded plots than just adult plant plots in 2017 after declines in 2016). The difference in bottom cover between plots with and without seeds was smallest at the beginning of the study, before any disturbances, and in years and locations where environmental conditions favoured high clonal survival. These results support models hypothesizing the pivotal role of sexual reproduction for population recovery after extreme die-off events (Greve, Krause-Jensen, Rasmussen, & Christensen, 2005; Jarvis, Brush, & Moore, 2014; Plus et al., 2003) but also potentially for augmenting the recovery from seasonal diebacks of varying severity. Previous genetic studies in the Baltic Sea recorded increased occurrences of new genotypes within experimentally disturbed *Z. marina* plots but also recorded consistent background occurrences of new genotypes in undisturbed plots, suggesting recruitment consistently occurred (Reusch, 2006). Intermediate or minor disturbances may potentially lower intraspecific competition precluding seedling recruitment among adult shoots and enhance seedling survival (Yang, HilleRisLambers, & Ruesink, 2016). In fact, disturbances of intermediate intensity were linked to increases in clonal richness for a suite of tropical seagrasses, whereas disturbances of high intensity associated with cyclones resulted in far lower clonal diversity (McMahon et al., 2017). In our study, seeded transplant plots within Spider Crab Bay never approached the bottom cover attained within adult plant plots. In this more energetic site, disturbance likely prevented seedling establishment and favoured the growth and survival of established clones over the emergence of seedlings. Our findings, in combination with previous genetic surveys of clonal richness, support the hypothesis that disturbance at some level to an existing population of seagrasses may open opportunities for seedling recruitment and therefore increase population genetic diversity. The reproductive plasticity of *Z. marina* and other seagrasses suggests a general pattern of recruitment for a given seagrass species likely does not exist, but that individual

populations of seagrasses likely adapt reproductive patterns, whether ISR or RSR (Eriksson, 1993), capable of maintaining and recovering populations in the face of disturbances.

Observations of seedling establishment and the increased importance of sexual reproduction at locations and in years with low adult cover suggest a reproductive 'bet hedging' strategy within the perennial *Z. marina* meadows of Chesapeake Bay. During years of high disturbance, for example, years with high summer water temperatures and/or poor water quality, low numbers of adult shoots will survive, and seedlings germinating after environmental conditions improve will likely constitute more of the shoots the following spring (Jarvis et al., 2014; Jarvis & Moore, 2010). During years with less seasonal disturbance, for example, years with cooler summer water temperatures and/or better water quality, more adult shoots can survive throughout the entire year and the relative influence of seedlings will likely be low compared to the growth of existing adult shoots the following spring (Moore et al., 2012). During these years, shoots of existing plants likely outcompete seedlings germinating within the meadow. The relative role of sexual reproduction in meadow maintenance would thus change along a gradient of disturbance to the existing canopy so long as seed production and environmental conditions suitable for seedling establishment exist.

Such selection for reproductive strategies to maintain populations likely exists more broadly. Sexual reproduction may serve an important role in *Z. marina* population survival at locations with discrete seasonal or cyclical disturbances, such as ice scour (Robertson & Mann, 1984), seasonal light disruptions (Kim et al., 2014) or high temperatures (Jarvis et al., 2012; Santamaria-Gallegos et al., 2000; this study), to the existing population, but which subside or disappear by the time seeds germinate. Conversely, at locations without acute seasonal disturbances or a high potential for disturbance at some point during seedling establishment, asexual reproduction may play a larger role in maintaining individuals and populations (Billingham et al., 2003; Reusch et al., 1999). The variability and importance of sexual reproduction in this study and across the distribution of *Z. marina* suggests the plasticity of reproductive traits among *Z. marina* populations may facilitate the species' colonization of diverse environments and habitats and enhance its resilience to natural and anthropological environmental stresses.

4.2 | Potential impact of seed supply and establishment

For sexual processes to accelerate meadow recovery from disturbances, seed production and banks must be sufficient at locations where a disturbance occurs and the disturbance itself cannot also disturb the seed or seedling bank. Flowering intensity and seed bank densities fluctuate in both space and time (Harwell & Orth, 2002; Phillips et al., 1983; Silberhorn et al., 1983; Van Lent et al., 1995). Results from seed addition experiments in our study suggest seed

availability or supply may influence the number of established seedlings. As a result, seedling recruitment may not reliably fill gaps that disturbances open in *Z. marina* canopies. In addition, for perennial *Z. marina* populations within the lower Chesapeake Bay and elsewhere, seedlings will generally flower for the first time in their second year of growth (Jarvis & Moore, 2010; Orth & Moore, 1983; Setchell, 1929). Multiple acute disturbances, for example, consecutive years of highly stressful summers, may thus dramatically reduce the flowering population and seed supply. With simultaneous low shoot survival and low seed supply, seagrass populations will struggle to recover (Jarvis et al., 2014; Jarvis & Moore, 2010; Kuusemäe et al., 2018; Valdemarsen, Wendelboe, Egelund, Kristensen, & Flindt, 2011). Similarly, if seed-based restoration were to remove a high proportion of seeds and lower the supply of seed available to the donor meadow, the donor meadow could experience lower seedling establishment. A previous study, however, did not recorded significant impacts from seed harvesting on donor meadows, likely because the adult population did not experience any disturbance after the harvesting, the removal did not significantly lower seed supply, the donor meadow was selected for its high fecundity or dispersal from adjacent populations still provided adequate seed supply (Marion & Orth, 2010). Meadows of *Z. marina* in Chesapeake Bay generally produce high densities of flowering shoots, so results from that study may not be applicable to regions where flowering shoot densities are much lower or flowering populations more isolated.

The intensity and timing of disturbances may also alter the relative benefit of sexual or asexual reproduction. Unlike in the York River, bottom cover within the seeded plots in Spider Crab Bay transplant garden experiments was consistently lower than in plots with adult transplants. The lower cover in seeded plots in Spider Crab Bay likely resulted from consistently higher wave and tidal current energy at this location relative to the York River during seedling establishment (Figure S4). Colder and clearer water within coastal lagoons of the Delmarva Peninsula may also favour shoot survival and asexual reproduction in Spider Crab Bay relative to the York River location (Moore et al., 2012). The combined impact of higher energy during vulnerable seedling establishment periods and higher water quality may select for higher clonal than seedling survival in Spider Crab Bay. Of course, disturbances during seed settlement or seedling establishment, even if away from competing adult vegetation, may also diminish the role of sexual reproduction in meadow maintenance (Marion & Orth, 2012; Yang et al., 2016). Should disturbances increase in frequency and intensity before or during seed production or seedling establishment, the resilience of seagrass populations reliant on sexual reproduction may decline and population collapse may occur.

4.3 | Impact of adult shoots on seedlings

Although previously hypothesized (Olesen, 1999; Olesen et al., 2017), our study observationally and experimentally demonstrates a relationship between surrounding vegetation and seedling survival within

existing meadows. Adult shoots do compete with each other and seedlings for common resources, such as space, light or nutrients (Gopal & Goel, 1993; Gustafsson & Boström, 2013; Invers, Zimmerman, Alberte, Pérez, & Romero, 2001; Williams, 1987). Interestingly, we observed the site with the lowest measured LAI, Sandy Point, was smothered in extensive and unexpected algal mats during the summer of 2017 (Figure S3). For this location, interactions with macroalgae, not surrounding seagrass shoots, likely compromised seedling survival. Although resource competition, whether inter or intraspecific, may not immediately or directly threaten seedling survival, the lower resource levels left for seedlings after adults draw down common resource pools can negatively impact seedling growth (Bintz & Nixon, 2001; Robertson & Mann, 1984; Zhang, Zhang, Niu, Sun, & Tian, 2014; Figures S5 and S6). For *Z. marina* in Chesapeake Bay near the southern limit of its distribution, growth during optimal environmental conditions is important for accumulating non-structural carbohydrate reserves needed to survive during stressful environmental conditions (Burke et al., 1996). Because seeds germinate in late fall in Chesapeake Bay and have the potential to grow in gaps before interacting with adult shoots in the spring, the timing and growth of seedlings prior to experiencing stressful summer environmental conditions could be critical to their survival and should be investigated more thoroughly (Orth & Moore, 1983; Figures S7 and S8). By slowing growth and the accumulation of reserves, resource competition could dramatically hamper seedling survival for *Z. marina*, as observed here, but also potentially other seagrass seedlings growing among established clones of their own or different species in other systems.

Some seedlings did, however, survive the stressful summer period at two of the locations studied. This survival suggests competition among adult clones does not entirely preclude seedling recruitment in perennial populations of *Z. marina*. The majority of the surviving seedlings were growing near Goodwin Island. This location maintained the second lowest estimated LAI of *Z. marina*, suggesting intraspecific competition at this location may have been lower than at other locations. In addition, seedlings at Goodwin Island were much closer to canopy height than at all other locations. The lower LAI and smaller difference in height between seedlings and adults of *Z. marina* at Goodwin Island may not have generated strong intraspecific competition for resources on vulnerable seedlings as at other studied locations. This result suggests seedling recruitment could occur for other seagrass species in locations or years where intraspecific or interspecific competition between adult shoots and seedlings is, if only temporarily, lower. Even the rare survival of a small number of seedlings, and thus the introduction of novel genets, could have profound evolutionary effects on a population.

5 | CONCLUSIONS

The relative role of sexual reproduction in seagrass meadow maintenance is likely a function of both seed supply and the survival of propagules competing with surrounding clones. Seedling establishment is an important demographic process within the perennial *Z. marina* meadows of our study system but varies across locations. Seed availability may partially explain this variability in establishment among locations. Interactions between surrounding shoots and established seedlings appear to limit the survival of seedlings within existing meadows through the stressful summer period in our system. Sexual processes will likely be important for *Z. marina* and other seagrass population dynamics where seed production is high and clonal survival is low at some point in either space or time, due to biotic (e.g. ray or crab holes, macroalgal mats) or abiotic (e.g. stressful water temperatures, ice scour or strong wave energy) disturbances. These disturbances to the population may provide windows of opportunity for seedlings to germinate and survive, so long as they are offset in time from seed germination and seedling development. Sexual reproduction and seedling recruitment may thus be required to play an increasingly important role in the persistence and resilience of seagrass populations disturbed by increasing anthropogenic pressures. Finally, these processes may only become more important to understand as efforts to restore seagrass begin to incorporate seed-based approaches.

AUTHORS' CONTRIBUTIONS

A.J.J., R.J.O. and K.A.M. developed the experimental design and methodology, contributed to fieldwork, wrote and edited the manuscript.

DATA AVAILABILITY STATEMENT

Data used in this publication is arched within the Dryad Digital Repository: https://doi.org/10.5061/dryad.2bvq83bm5 (Johnson, Orth, & Moore, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.